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Abstract

Epithelia cover the surfaces and line the cavities of the body. Recent studies have highlighted the existence of multiple stem cell compartments within individual epithelia that exhibit striking plasticity in response to tissue damage, transplantation or tumour development. New knowledge about the composition of the epithelial niche, and the transcription factor networks that maintain cell identity have provided new insights into the extrinsic and intrinsic regulation of stem cell behaviour. In addition new *in vitro* tissue substitutes allow better integration of data from human and mouse models.

Introduction

Epithelia are one of the four tissue types of the body, the others being connective tissue, muscle and nerve. Epithelia form the body's interface with the environment and line body cavities, such as the lumen of the intestine. A hallmark of epithelia is that they are constituted of sheets of cells that are tightly packed via specialized cell-cell junctions and adhere to a specialized extracellular matrix, known as the basement membrane (Watt and Fujiwara, 2011). Some epithelia, such as the pulmonary epithelium of the lung, comprise a single cell layer (simple epithelium), while others, such as the lining of the mouth, consist of many cell layers and are known as stratified epithelia. In addition, epithelia may contain adnexal structures, such as glands or hair follicles, which have specific functions.

Epithelia are very heterogeneous (Figure 1). In the skin, the epidermis prevents body dehydration, protects against radiation and penetration by microorganisms and withstands mechanical stress. Simple epithelia that interface the external environment, such as the lung and the intestinal epithelia, also act as a barrier against pathogens in addition to hosting commensal bacteria. Examples of epithelial cells with specific secretory functions are epidermal sebocytes, which release lipids to lubricate the skin surface, and intestinal Paneth cells, which release anti-microbial compounds that are important in host immunity. While Paneth cells are distributed individually within the crypts of the small intestine, sebocytes are organized in glands associated with the hair follicles. In stratified epithelia the stem cells are attached to the basement membrane, while in simple epithelia the differentiated cells, like the stem cells, are attached to the basement membrane.

The epidermis and intestine are two of the tissues in which the existence of stem cells was first postulated. This is because the terminally differentiated cells are unable to divide and must be replaced throughout adult life by less differentiated cells, the stem cells, that both self-renew and generate differentiated progeny (Hall and Watt, 1989). In other epithelia, such as the lung and mammary gland, the kinetics of cell differentiation and replacement are

somewhat different. For example, in mammary gland periods of dormancy are interspersed with phases of hormone-driven morphogenesis, at puberty and during pregnancy (Visvader and Stingl, 2014). The rate of cell turnover is not only highly variable between different epithelial tissues - ranging from a few days in the small intestine crypts to a few weeks in the epidermis and several months in the urinary tract epithelium – but also varies within a single epithelium, with some cells dividing much more rapidly than others. This complexity highlights the tight regulation of stem cell activity required to maintain tissue homeostasis.

In this article we discuss recent advances in our understanding of epithelial stem cells and the intrinsic and extrinsic factors that regulate their behaviour. Within each epithelium multiple stem cell populations are physically and functionally compartmentalised, yet they can exhibit striking plasticity when perturbed. Clonal analysis has revealed epithelial stem cells and their progeny to exhibit dynamic behaviour, which reflects in part responses to extrinsic signals from the stem cell niche and in part intrinsic control of cell state via transcriptional and epigenetic regulators. The integration of single cell data with in vivo lineage tracing is increasing our understanding of epithelial stem cell properties, while the generation of epithelia from pluripotent stem cells is blurring the traditional boundary between pluripotent and tissue stem cells.

Individual epithelia contain multiple stem cell populations

Over the years a range of *in vitro* and *in vivo* assays have been developed to identify and quantify epithelial stem cells (Kretzschmar and Watt, 2012). Of these, lineage-tracing experiments represent the most powerful method to study stem cells under homeostatic conditions. Not only does lineage tracing avoid the need to disaggregate the tissue to identify stem cells, but it also allows visualization of the contribution of stem cell progeny to multiple differentiated cell types, which are often organized into complex adnexal structures such as the hair follicles of the skin.

One important principle to emerge from lineage tracing in multiple epithelia is that of stem cell compartmentalization. It is evident that the intestine, mammary glands, lungs and epidermis are not obligatorily maintained by a

single, homogeneous, stem cell population. Rather, there are several stem cell pools, characterized by differences in the molecular markers they express and their context-dependent functions. For example, in mouse skin (Figure 2A, B), stem cell populations have been identified in the interfollicular epidermis, hair follicle bulge, hair follicle junctional zone and sebaceous gland. In the lung, lineage tracing of Scgb1a1+ Clara cells suggests a model in which trachea, bronchioles, and alveoli are renewed by distinct epithelial stem cells during adult homeostasis (Rawlins et al., 2009). In mouse esophageal epithelium there are basal cell subpopulations with distinct differentiation and proliferation properties, consistent with a hierarchical model that involves stem cell progeny differentiating in the basement membrane niche (DeWard et al., 2014).

In the intestinal epithelium *Lgr5* expressing stem cells give rise to multiple differentiated progeny, including absorptive enterocytes, goblet, Paneth and enteroendocrine cells (Barker et al., 2007) (Figure 2C). Although several studies have indicated the existence of stem cell heterogeneity (Sangiorgi and Capecchi, 2008; Takeda et al., 2011; Li and Clevers, 2010; Itzkovitz et al., 2012; Munoz et al., 2012)), live imaging has recently established that Lgr5-positive cells are at the top of the cellular hierarchy in the intestine. Nevertheless, cells located above the crypt base can occasionally revert to the bottom of the crypt following cell division (Ritsma et al., 2014). (Itzkovitz et al., 2012; Munoz et al., 2012) (Figure 2D).

While the stem cells of individual epithelia are undoubtedly heterogeneous, some stem cell markers are common to multiple epithelia. This has become evident from the development of transgenic mice expressing Cre recombinase under the control of specific gene promoters. One of the first examples was *Lgr5*, originally found as a stem cell marker in the small intestine, then subsequently found to mark stem cell populations in the lower hair follicle, mammary gland and stomach (Barker et al., 2007; Jaks et al., 2008). *Lrig1* is also expressed in stem cells of a variety of epithelia, and there is partial overlap of expression between *Lgr5* and *Lrig1* (Powell et al., 2012). It is likely that the existence of common stem cell markers in different epithelia reflects the fact that common pathways regulate the stem cell compartments, including *Wnt*, *Egf* and *Notch* (Estrach et al., 2008; Jensen and Watt, 2006; Lim et al., 2013; Schuijers et

al., 2015; van Es et al., 2012). For example, Lgr5 is a Wnt target gene and Lrig1 is a negative regulator of Egf signalling.

Quantitative analysis of epithelial clones reveals dynamic stem cell behaviour in healthy tissue and in cancer

Statistical analysis of the size and distribution of epithelial cell clones marked by lineage tracing has given new insights into the behaviour of epithelial stem cells. Clone size is relatively easy to quantify in epithelia, because intercellular adhesive junctions maintain stem cell progeny in proximity to one another. In mouse tail epidermis, inducible genetic labeling first allowed long term analysis of the fate of individual cells. Clone size distributions suggested that only one type of cell maintains epidermal homeostasis through both symmetric and asymmetric division (Clayton et al., 2007). This view was subsequently challenged by clonal analysis of cells expressing K14, a epidermal basal layer marker and *Involucrin*, which is primarily expressed by differentiating cells. The analysis suggested that slow-cycling *K14*-expressing stem cells give rise to *Involucrin*-positive transit-amplifying cell progeny (Mascre et al., 2012). In fact the situation is more complex because neither study took into account the existence of two distinct differentiation programs within tail interfollicular epidermis, which are maintained by discrete cell populations in the basal layer (Gomez et al., 2013). At birth the tail epidermis comprises a single differentiated lineage, but from P9, a second type, known as the scale lineage, is established. The scale expands by more rapid proliferation of the stem cell compartment than that of the interscale, and the relative size of each compartment controlled by Wnt and Lrig1 signalling (Gomez et al., 2013).

Long-term fate mapping of clones in intestinal epithelium using the R26R-Confetti allele has revealed that stem cells compete to colonize each crypt following neutral stochastic drift dynamics. Intestinal crypts become monoclonal within one to six months, indicating that there is a natural predisposition toward fewer but larger clones (Ritsma et al., 2014; Snippert et al., 2010) (Figure 3A). Live imaging has shown that individual Lgr5 expressing stem cells at the base of the crypt have a positional advantage in self-renewal ability compared to stem cells in the upper part of the crypt, since dividing cells can push neighbouring

stem cells out of the niche (Ritsma et al., 2014). Multicolor fate mapping in mammary glands suggests that, over time, a few stem cell clones prevail and colonize large areas of the epithelium in a similar manner to the intestine (Rios et al., 2014).

One situation in which lineage analysis has been performed in human tissue is in the intestinal crypt, where somatic mtDNA mutations allow tracing of clonal lineages. This has shown that the clonal evolution of human intestinal stem cells resembles that of the murine crypt and although human crypts house 10 times more cells than mouse, both human and mouse crypts have a similar number of stem cells (Baker et al., 2014).

Mathematical modeling and statistical analysis of *in vivo* stem cell behavior has not only provided new insights into epithelial homeostasis but can also aid interpretation of the consequences of perturbing homeostasis (Blanpain and Simons, 2013). For example, inhibition of the Notch pathway in esophageal epithelial stem cell clones generates an imbalance in neutral drift dynamics in favor of positive selection of mutant clones at the expense of wild type clones. This is the result of an increase both in the proliferation rate of the mutant stem cell clones and in their ability to accelerate neighbouring wild-type cell differentiation (Alcolea et al., 2014). A similar effect is observed when oncogenic K-ras is sporadically activated in the intestinal LGR5 stem cells. Cell proliferation increases, creating a biased drift towards crypt clonality (Snippert et al., 2014).

Another example of clonal perturbation occurs in “field cancerisation”: in the early stages of tumor development an unequal competition between normal and mutant cells leads to clonal expansion of histologically normal but genetically altered cells. Indeed clonal selection is a key process in tumor growth; cancer evolves through the expansion of the most successful genetically altered clones that are able to adapt and proliferate in tumor niches (Figure 3B). The analysis of changes in the size distribution of *p53* mutant clones in chronically UVB-irradiated mouse epidermis has revealed that, as in normal epidermis, the fate of individual clones is stochastic, resulting in a balance between cell loss and proliferation. Preneoplastic clones are not derived from long-lived mutant stem cells but from mutant progenitors with a random fate (Klein et al., 2010). In mouse intestine the fate of stem cells that have a potential

competitive advantage through Apc loss, Kras activation and P53 mutation has been quantified. Surprisingly, many of the mutant stem cells are stochastically replaced by wild-type stem cells; only P53 mutations provide a competitive advantage (Vermeulen et al., 2013). These studies raise the interesting question of whether the drift towards monoclonality that is characteristic of normal epithelia increases the likelihood that mutant clones will expand to become tumors (Figure 3B).

Lineage tracing has provided evidence for the existence of cancer stem cells in epithelial tumors. By definition, cancer stem cells have the highest potential to propagate and maintain tumor growth when compared with other cells in a tumour and they represent the top of a cancer cell hierarchy (Figure 3B). In benign epidermal tumors, named papillomas, multiple genetically labeled tumor cells contribute to the bulk of the tumor; however in malignant squamous cell carcinomas, clonal analysis indicates that tumour formation reflects expansion of individual cancer stem cells (Driessens et al., 2012). In mouse intestine, Lgr5 cells that represent 5-10% of all tumor cells are responsible for the growth of established adenomas (Schepers et al., 2012). Lineage tracing has also shown the existence of a small population of cancer stem cells in mammary tumors, although intravital imaging has recently highlighted the dynamic nature of these cells during tumor growth (Zomer et al., 2013).

Epithelial stem cells exhibit plasticity during tissue repair and regeneration

In addition to the concept that individual epithelia harbor multiple stem cell populations (stem cell heterogeneity), a second principle that has emerged is that individual epithelial stem cell populations exhibit wider differentiation potential in tissue reconstitution assays than in the intact tissue (stem cell plasticity; Figure 2A, B; Figure 4). This has been shown for the epidermis, intestinal epithelium, lung and mammary gland (Van Keymeulen et al., 2011). For instance *Lrig1* positive epidermal stem cells give rise to differentiated cells that are restricted to specific areas of the pilosebaceous unit under steady state conditions, but in skin reconstitution assays *Lrig1* positive cells contribute to all the different epidermal compartments (Jensen et al., 2009; Page et al., 2013)

(Figure 4A). Even in the hematopoietic system, where serial reconstitution of the blood by individual cells has long been the gold standard for stem cell identification (Rossi et al., 2008), it is reported that cells behave differently under steady state conditions. This was demonstrated by native non-transplant hematopoiesis by cell labeling in situ through inducible transposon tagging (Sun et al., 2014) and by inducible genetic labelling of Tie2+ haemopoietic stem cells in bone marrow (Busch et al., 2015).

While cell transplantation assays have revealed a broader plasticity of epithelial stem cells than they exhibit under steady state conditions (Prater et al., 2014; Van Keymeulen et al., 2011), it could be argued that this is of limited physiological relevance. A single mammary gland cell will never normally be found in isolation in a cleared mammary fat pad, and the skin is never normally reconstituted from a mixture of disaggregated epidermal and dermal cells. Nevertheless, the plasticity revealed by those studies is also seen during tissue repair (Figure 2, 4A). In the hair follicle, Lgr5 and Lrig1-expressing epidermal stem cells produce differentiated progeny that are normally restricted to the hair follicle. However, following skin wounding their progeny are able to migrate into the interfollicular epidermis where they persist for long periods (Ito et al., 2005; Jaks et al., 2008; Page et al., 2013) (Figure 2A, B). Even targeted laser ablation of individual stem cells can provoke a change in cell fate, as in the hair follicle, where cells that have left the stem cell compartment are able to dedifferentiate and become bulge stem cells (Rompolas et al., 2013).

In the small intestine genetic ablation of Lrg5-positive stem cells does not perturb epithelial homeostasis because of compensatation by a different stem cell pool comprising Bmi1-expressing cells (Tian et al., 2011). Dll1-expressing cells that are committed to the secretory lineage can revert to an intestinal stem cell state after tissue damage (van Es et al., 2012). Similarly it has been show that mouse intestinal quiescent cells that are committed precursors of secretory Paneth cells and enteroendocrine lineage, can give rise the main epithelial cell types after intestinal injury (Buczacki et al., 2013). In the lung, type II pneumocyte progenitors mediate highly focal lung repair, while p63/Krt5-

positive distal airway stem cells are essential for lung regeneration after acute lung damage (Hogan et al., 2014; Zuo et al., 2015).

The interaction between neighboring epithelial cells in different differentiation states can drive stem cell plasticity. In the epidermis, by promoting Shh signaling cells that have exited the stem cell compartment directly stimulate quiescent stem cells to expand and indirectly, via dermal factors, favor their own proliferation (Hsu et al., 2014). When airway stem cells in the lung are ablated, luminal secretory cells can de-differentiate into basal stem cells that are multipotent and have the ability to repair the epithelium. This de-differentiation process is normally inhibited by cell-cell interactions with basal stem cells (Tata et al., 2013).

Wound healing repairs tissue without necessarily restoring full functionality. In contrast, regeneration is a process that leads to a full recovery of tissue functions. Although adult mammals cannot regrow entire amputated limbs, the distal tips of the digits in mice can be regenerated. The process involves coordinated changes in a wide range of different cell types and stem cell populations without conversion between different lineages (Rinkevich et al., 2011). In the nail, basal epidermal stem cells orchestrate the regeneration process through *Wnt* activation (Takeo et al., 2013).

One emerging concept is that the nature of an injury can determine whether or not tissue is fully regenerated in addition to being repaired. For instance, the excision of a small area of adult mouse skin is repaired through a wound healing process leading to scar formation without de novo formation of hair follicles. However the excision of a large area of skin leads to *de novo* hair follicle regeneration as a result of Wnt activation (Ito et al., 2007). A broad concept that is starting to emerge is that the changes in cell state that occur in response to injury, and therefore the degree to which tissue function is regenerated, depend on the nature and extent of the injury. This could, for example, resolve the current controversy about the cell types that are responsible for liver repair (Grompe, 2014; Schaub et al., 2014; Tarlow et al., 2014).

The process of transdifferentiation – conversion from one lineage to another – is a well-documented developmental process. For example, during

tooth development a significant proportion of mesenchymal stem cells are derived from peripheral nerve-associated glial cells (Kaukua et al., 2014). However, in addition, epithelial cells can undergo transdifferentiation during tissue repair. In adult pancreas, loss of beta cells can lead to transdifferentiation of acinar cells into beta cells (Thorel et al., 2010). During liver regeneration, hepatocytes can be converted into biliary epithelial cells (Yanger et al., 2013).

Transdifferentiation also occurs in the context of disease, in particular the phenomenon of metaplasia: the replacement of a normal cell type with another differentiated cell type. This phenomenon, although rare, affects multiple epithelia and can predispose to cancer development (Slack, 2007). For example, Barrett's esophagus is a condition in which the normal esophageal stratified squamous epithelium is replaced by a simple columnar epithelium containing goblet cells that are normally found in the intestinal tract. Although this is a reversible process, it is associated with development of esophageal cancer. A further example is in breast cancer, where transdifferentiation of mammary epithelial cells into squamous epithelial carcinoma cells reflects a transition from a simple mammary epithelium to a stratified epithelium (Li et al., 2003).

The existence of cancer stem cells and the evolution of cell clones by genetic mutations represent two well-established and interconnected features of cancer (Figure 3B). We hypothesize that a third mechanism that could contribute to clonal expansion and heterogeneity is via acquisition of the same type of cell plasticity as observed during repair of healthy tissue in absence of genetic mutational events (Figure 3B). This begs the question: what is the nature of stem cell plasticity? At present we do not know whether, within a given epithelium, different stem cell types share a common state of plasticity, and whether the stem cells of a tissue in which homeostasis has been re-established are the same as, or different from, the stem cells of the undamaged tissue (Figure 5).

Fibroblasts, immune cells and bacteria are dynamic constituents of the stem cell niche

It is well established that the stem cell microenvironment, or niche, regulates many aspects of cell fate. Several generic constituents of the niche, including

extracellular matrix composition, physical forces and neighbouring cells, are well known to affect epithelial stem cell behaviour (Lane et al., 2014). In addition, three other aspects of the niche are becoming better understood. These are connective tissue fibroblasts, cells of the immune system and the microbiome. These niche components help to maintain the functional diversity of epithelial stem cells during homeostasis, during tissue repair and in cancer (Figure 6).

In skin, different mesenchymal cell types regulate epidermal stem cell properties. These include the adipocyte lineage, which positively regulates hair follicle stem cell activity (Festa et al., 2011) and the cluster of cells at the base of the hair follicle, known as the dermal papilla, which contribute to maintaining hair follicle stem cells identity. Recent studies have shown that different subsets of skin fibroblasts arise from different lineages during development. The upper dermal lineage not only gives rise to the dermal papilla, but also to the arrector pili muscle and papillary fibroblasts. The lower dermal lineage gives rise to reticular fibroblasts involved in the production of the fibrillar extracellular matrix and the adipocyte lineage. The upper lineage is required for hair follicle formation, while the lower lineage mediates the first wave of dermal repair following wounding (Driskell et al., 2013). Epidermal *Wnt* signalling regulates the different dermal compartments (Driskell et al., 2013), by a combination of short (Fujiwara et al., 2011) and long (Donati et al., 2014) range signalling.

The stromal cells of mouse lung also comprise different cell lineages, with different populations being organized into localized and tightly regulated domains within the tissue. As in the epidermis, the different fibroblast subsets regulate epithelial proliferation and reciprocal communication is mediated by Wnt signalling (Kumar et al., 2014). Therefore, the picture that emerges both in the epidermis and the lung is that the mesenchyme is just as complex and compartmentalised, in terms of cellular heterogeneity, as the epithelium.

Fibroblast heterogeneity does not only contribute to maintaining epithelial heterogeneity during homeostasis but also contributes to cancer progression, since tumor stroma is a key factor in cancer initiation, growth and progression. Fibroblast secretion of extracellular matrix proteins, such as fibrillar collagens, influences the mechanical properties of connective tissue. In the mammary gland increased matrix stiffness can promote tumor progression

through microRNA-dependent PTEN expression (Mouw et al., 2014), suggesting a role for the fibroblast lineages responsible for ECM deposition. The isolation of cancer-associated fibroblasts (CAFs) from different stages of breast cancer progression has shown that CAFs require the transcription co-activator YAP to promote matrix stiffening, cancer cell invasion and angiogenesis (Calvo et al., 2013). Other signalling pathways and transcription factors that regulate the properties of tumour stroma have also been identified. In skin UVA light downregulates stromal Notch signaling, and mesenchymal-specific deletion of a key Notch effector triggers stromal atrophy, inflammation and field cancerisation (Hu et al., 2012). The transcriptional regulator heat shock factor 1 (HSF1) is frequently activated in CAFs. HSF1 expressing CAFs support malignancy in a non-cell-autonomous way secreting TGF-β and SDF1 (Scherz-Shouval et al., 2014). While the signalling pathways that mediate the contribution of CAFs to cancer are increasingly well characterised, the possibility that CAFs are heterogeneous rather than a uniform cell population ,is only just starting to emerge (Ohlund et al., 2014; Sugimoto et al., 2006). The functional heterogeneity of fibroblasts observed in healthy epithelia also contributes to the heterogeneity of CAFs in melanoma (Rinkevich et al., 2015).

Cells of the immune system are key components of epithelia, both in health and disease (Pasparakis et al., 2014). In the epidermis γδ T cells influence survival, proliferation and migration of keratinocytes, and represent a crucial component for hair follicle neogenesis after skin wounding, indicating their role in the epidermal stem cell niche (Gay et al., 2013). As in the case of epithelial-fibroblast communication, communication with immune cells is reciprocal. For example, adult thymic epithelial cells regulate the fate of hematopoietic cells, facilitating the selection of thymocytes with functional T cell receptors (Anderson and Takahama, 2012).

Finally, while it is well known that as part of their barrier function epithelia are colonised by commensal bacteria, it has only recently become apparent that their impact can be on specific epithelial stem cell compartments, particularly in the lung, the skin and intestine. This is because different microbiota colonise different sites within an epithelium, dependent on environmental factors such as temperature and humidity (Grice and Segre,

2011). The interdependence between epithelial tissue functionality and specific microbiota has been identified. In early life, lung microbiota promote tolerance to allergens. Following exposure to allergens, there is a positive correlation between an increased number of goblet cells and a change of microbiota at the phylum level (Gollwitzer et al., 2014). In the intestinal crypt, Lgr5+ stem cells express high levels of the cytosolic innate immune sensor Nod2. Nod2 stimulation protects against oxidative stress and promotes cell survival, suggesting that gut repair can be supported by the presence of bacteria (Nigro et al., 2014).

The presence of specific bacteria can also impact epithelial tumor formation. While this is well known in the case of the Helicobacter pylori and gastric cancer (Sigal et al., 2015), it also appears to be true in the skin. Chronic skin inflammation and wounding can trigger cancer and in a mouse model this has been linked to the presence of flagellated bacteria, which are recognised by Toll-like receptor 5 (Hoste et al., 2015). Pathogen surveillance involves a precise cascade of cellular events in epithelial tissue. For example in skin once Cd103 dendritic cells sense the presence of *Staphylococcus epidermidis*, they migrate to the lymph nodes where they prime Cd8 T-cells. Subsequently, commensal specific- Cd8 T-cells move to the skin where they enhance the antimicrobial defense of keratinocytes (Naik et al., 2015). Intriguingly, dermal adipocytes have recently been shown to participate in skin defense against bacteria, demonstrating that microorganisms are not solely part of the epidermal stem cell niche (Zhang et al., 2015).

Transcription and epigenetic factors regulate epithelial stem cell state

Stem cell identity and function depends on both extrinsic factors (components of the niche) and cell intrinsic factors.. The intrinsic regulators are sequence specific transcription factors and epigenetic factors controlling transcriptional networks to drive tissue- specific gene expression patterns. In embryonic stem cells so-called super-enhancers, consisting of large clusters of enhancers bound by chromatin and transcription factors, have been shown to define cell identity (Hnisz et al., 2013). Recently super-enhancers have also been characterized in adult epidermal cells. These TF-dense DNA regions are very dynamic, since they

are remodeled in response to microenvironmental changes, differentiation status and during wound healing (Adam et al., 2015).

In epithelia there are several examples of how loss of a single transcription factor can lead to stem cell fate alteration. In the epidermis LHX2 plays a critical role in organising the niche of hair follicle stem cells, balancing cell quiescence and lineage selection (Folgueras et al., 2013). Loss of the transcription factor Cdx2 leads to transformation of intestinal stem cells into gastric stem cells (Simmini et al., 2014). One explanation for the profound effects of individual transcription factors is that they represent a crucial outcome of cell signaling. In intestinal epithelium, the mechanism that links *Wnt* level with cell type specification involves the transcription factor Ascl2, which interprets a *Wnt* gradient and specifies stem cells by a bimodal switch (Schuijers et al., 2015).

Histone and DNA epigenetic modifications are also important regulators of epithelial stem cell differentiation. Changes in DNA methylation state are associated with the differentiation of hair follicle stem cells towards multiple lineages (Bock et al., 2012). Similarly, DNA methylation dynamics at the enhancers rather than in the proximity of transcription start sites, are detected during small intestine stem cell differentiation (Kaaij et al., 2013). A network of epigenetic factors including Ing5, Bptf, Smarca5, Ezh2 and Uhrf1 controls epidermal stem cell fate via a mechanism that includes the regulation of genes that encode proteins responsible for anchoring epidermal stem cells to the extracellular matrix (Mulder et al., 2012). Cbx4, a Polycomb Repressive Complex 1 associated protein, maintains epidermal stem cells in a slow-cycling and undifferentiated state and protects them from senescence (Luis et al., 2011). In addition, changes in chromatin dynamics allow hair follicle stem cells to cyclically change between quiescent and activated states without losing their stem cell identity (Lien et al., 2011). In the intestine, the mechanisms that allow changes in cell identity between stem cells and differentiated cells involve a permissive chromatin state (Kim et al., 2014).

Chromatin factors can differentially regulate distinct cell compartments within the same epithelium. In adult epidermis Jarid2 depletion results in delayed hair follicle cycling due to decreased stem cell proliferation, but does not affect the stem cells of the interfollicular epidermis (Mejetta et al., 2011).

Transcription and chromatin factors can locally cooperate to establish expression gene expression patterns that control stem cell fate. The transcription factor ZNF750, for example, interacts with multiple chromatin regulators to repress epidermal stem cell genes and induce differentiation (Boxer et al., 2014). The functional interaction of c-Myc and the histone methyltransferase Setd8 is required to maintain the sebaceous glands and interfollicular epidermis (Driskell et al., 2012).

Effects of some TFs and chromatin factors only become evident when epithelial homeostasis is disturbed. For example, ablation of the transcriptional co-activator YAP does not affect homeostasis in the intestinal epithelium, yet is required for intestinal epithelial repair after injury (Cai et al., 2010). In mammary glands *Ovol2* represses the EMT transcriptional program and is an essential player during tissue regeneration (Watanabe et al., 2014). In skin the PRC2-complex and its repressive histone marks are reduced during wound healing (Shaw and Martin, 2009), consistent with the role of the H3K27 methyltransferases *Ezh2* and *Ezh1* in wound repair (Ezhkova et al., 2009). These examples clearly show the importance of TFs and chromatin factors during tissue damage. However it remains to be uncovered how these factors contribute to epithelial cell plasticity (Figure 5) and whether super-enhancer dynamics are involved (Adam et al., 2015).

The analysis of key players in gene regulation has shown the existence of epigenetic and transcriptional regulators that balance the heterogeneity within an epithelium in term of differentiation state of cells and lineage identity within a specific epidermal compartment.

Understanding cellular heterogeneity at single cell resolution

One intriguing aspect of gene transcription is transcriptional noise, which is the variability in gene expression occurring between cells in the same population. Transcriptional noise can control cell fate in hematopoietic lineage selection (Chang et al., 2008) and the noise resulting from transcriptional fluctuations of the pluripotency genes *Sox2*, *Oct4*, and *Nanog* may maintain pluripotency by counteracting differentiation stimuli (Kalmar et al., 2009). Although analysis of cell populations has led to important advances in our knowledge of epithelial

stem cell biology, single cell resolution is required to understand whether phenomena such as transcriptional noise affect epithelial homeostasis (Figure 7).

One of the first attempts to understand gene expression by global gene expression profiling in individual epithelial cells identified Lrig1 as a stem cell marker and showed that it regulated stem cell quiescence (Jensen and Watt, 2006). Subsequent studies have indicated the existence of two different states of human epidermal stem cells that have not been detected by conventional approaches. Nevertheless, whether they represent distinct cell lineages or transitional, interconvertible, cell states remains to be explored (Tan et al., 2013).

Single cell transcriptome analysis in intestinal epithelial cells has shown that the widely used reporters consisting of CreER and EGFP driven by the Lgr5, Bmi1 and Hopx, promoters often mark heterogeneous populations and, in addition, that there are discrepancies between reporter activity and endogenous transcripts. This analysis has also highlighted that Lgr5, Bmi1 and Hopx cell populations are molecularly and functionally distinct, supporting a role for two distinct stem cell populations in intestinal maintenance (Li et al., 2014). By combining single cell (genomic and proteomic) approaches with *in vivo* fate mapping it is possible to gain an understanding of stem cell dynamics at higher resolution than by lineage tracing alone, as illustrated recently for lung epithelium (Treutlein et al., 2014)

Analysis of single cell data has been greatly enriched by the development of new computational methods. For instance by applying the Wanderlust algorithm to single-cell mass cytometry data, it was possible to construct trajectories of how hematopoietic stem cells differentiate into B cells, identifying new cell states and aligning them to specific signalling pathways (Bendall et al., 2014). To robustly identify previously hidden cell subpopulations, computational methods can eliminate the effects of potential confounding factors (such as cell cycle stage) on the heterogeneity of gene expression identified by single cell RNA sequencing (Buettner et al., 2015). Single cell RNA-seq data have been also analyzed using the self-organizing map (SOM), which represents a way to visualize and interrogate cell-to-cell heterogeneity based on the behavior of coordinately expressed gene clusters (Kim et al., 2015).

The high throughput data provided by RNA-seq experiments lacks in positional information. This is not a problem with blood cells in the circulation, but is a major disadvantage for analysing epithelial cells, since spatial organization often reflects cellular heterogeneity. In order to overcome this problem a recent method called FISSEQ (fluorescence in situ RNA sequencing) has been developed that combines single cell transcriptome analysis in a spatial context (Lee et al., 2014).

Generation of epithelial tissues from pluripotent stem cells

Techniques for generating epidermis in vitro date back many decades, but progress in regenerating other epithelia in culture is more recent. Important advances include techniques for growing epithelial organoids from single cells and recapitulating the differentiated lineages that are present in the normal tissue. It is possible to grow intestinal organoids from purified LGR5-positive stem cells, such that a single crypt can generate villus-like epithelial domains in which all differentiated cell types are present, in the absence of a non-epithelial cellular niche (Sato et al., 2009). Similar culture conditions allow growth of epithelial organoids from human small intestine and colon (Sato et al., 2011), which could be used as a resource for drug screening or to graft genotyped matched human intestine and colon epithelium. A 3D culture system to maintain mouse and human prostate organoids has also been developed (Karthaus et al., 2014). It is also possible to recreate salivary glands and hair follicles in culture (Nanduri et al., 2014; Tobin, 2011).

An extension of these studies is to generate epithelial tissues from human pluripotent stem cells. This offers the possibility of not only generating the epithelia themselves, but also associated cell types, such as melanocytes and fibroblasts in the case of skin (Bilousova and Roop, 2014). Progress is not restricted to skin, as gastric organoids have been successfully generated from pluripotent cells (McCracken et al., 2014). It is yet to be determined how similar the epithelia generated from pluripotent cells are to adult or fetal tissues, as immaturity is a concern for other differentiated cells types, such as liver (Stutchfield et al., 2010).

Looking forward

In the recent years we have gained unprecedented insights into the nature and regulation of stem cells in a variety of postnatal epithelia, finding common principles such as stem cell compartmentalisation and plasticity. Looking to the future, single cell genome-wide technologies will give us new perspectives on cell identity and the interplay between epigenetic and transcriptional regulation. Creation of entire epithelial organs from pluripotent stem cells will have practical applications in disease modelling and drug discovery, while modulating the stem cell niche via changes in stromal cells, immune cells and pathogens will improve the quality of tissue repair for medicine regenerative proposes.

Figures

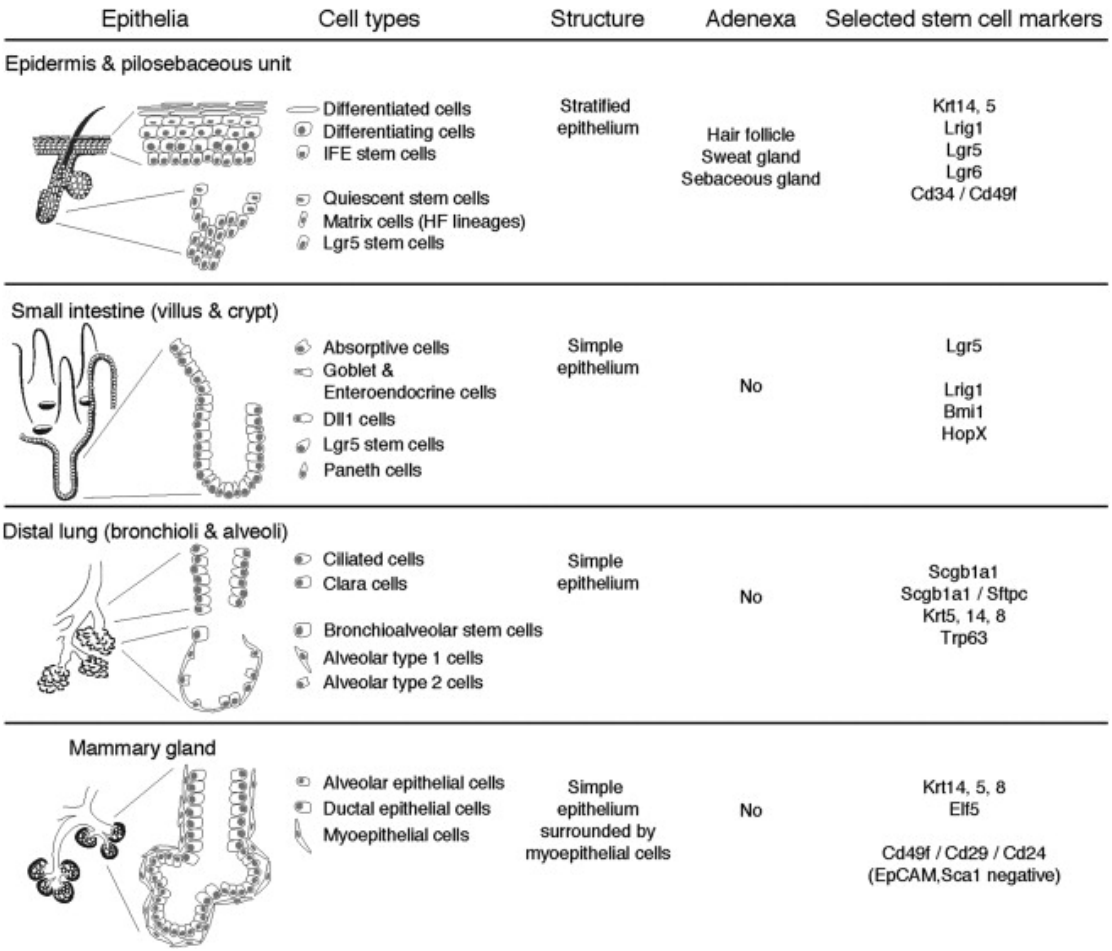

Figure 1. Different types of epithelia. Schematic summary of the architecture and stem cell compartments of different types of epithelia.

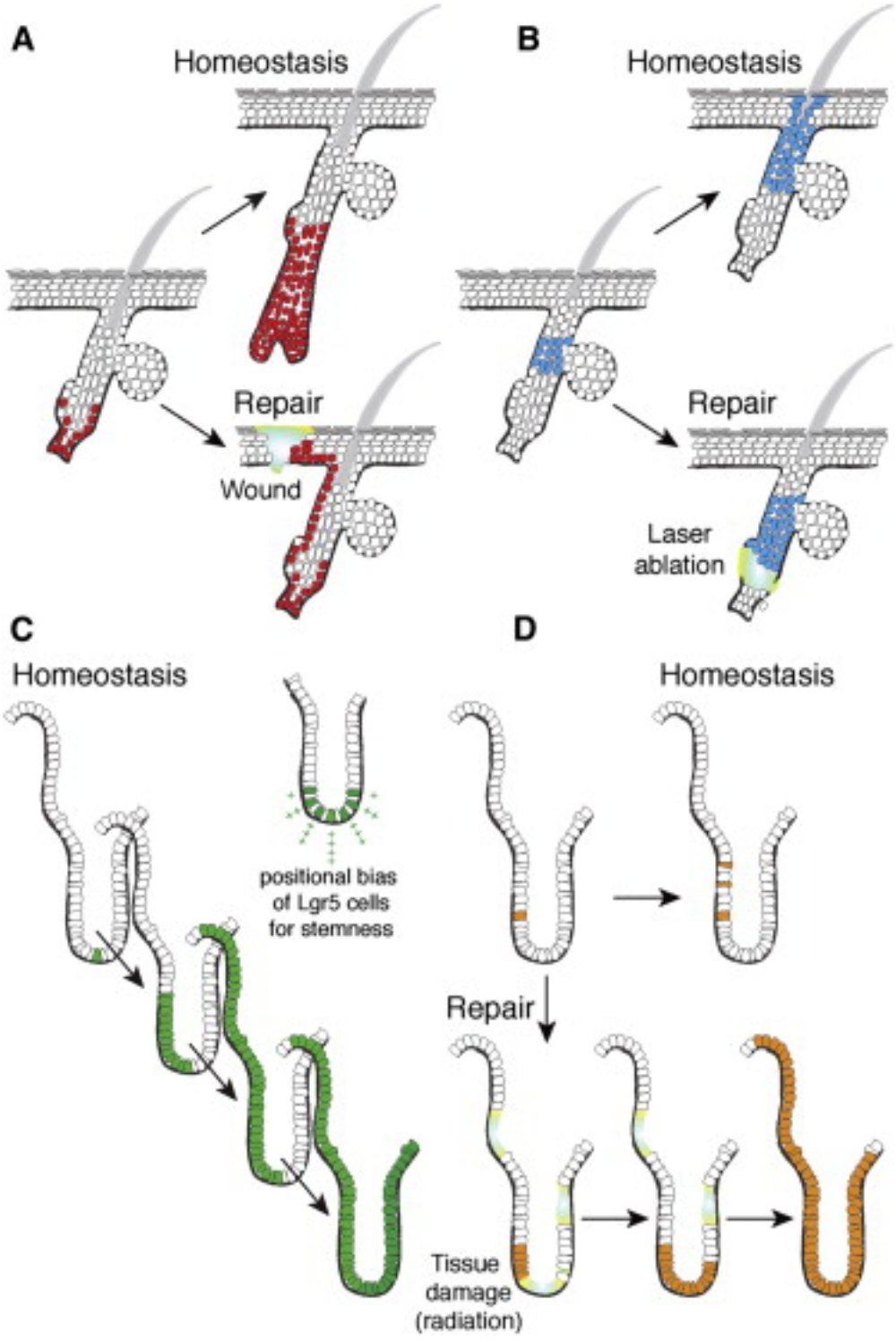

Figure 2. Epithelial stem cell contributions during tissue homeostasis and repair. (A, B) Epidermis; **(C, D)** intestinal epithelium**.** (A, B) Under steady state conditions (homeostasis) distinct epithelial stem cells give rise to distinct differentiated progeny. However, during repair of tissue damage they acquire plasticity and exhibit wider differentiation potential. (A) Lgr5-positive stem cells and their progeny (red), (B) Lrig1-positive stem cells and their progeny (blue)

Lgr5- and Lrig1-positive stem cell populations are located in separate HF compartments (lower bulge and isthmus, respectively) and in homeostasis they maintain the lower follicular bulb and the upper HF (infundibulum), respectively. During repair these restrictions are broken and they contribute to re-epithelialization more widely. (C) Lgr5-positive stem cells (green) at the base of the crypts give rise to all the lineages of the intestinal epithelium (left panel). (D) Dll1-positive cells (orange) are progenitors of the secretory lineages in homeostatic epithelium but. upon crypt damage Dll1 cells can revert to Lgr5 stem cells and repopulate the entire crypt..

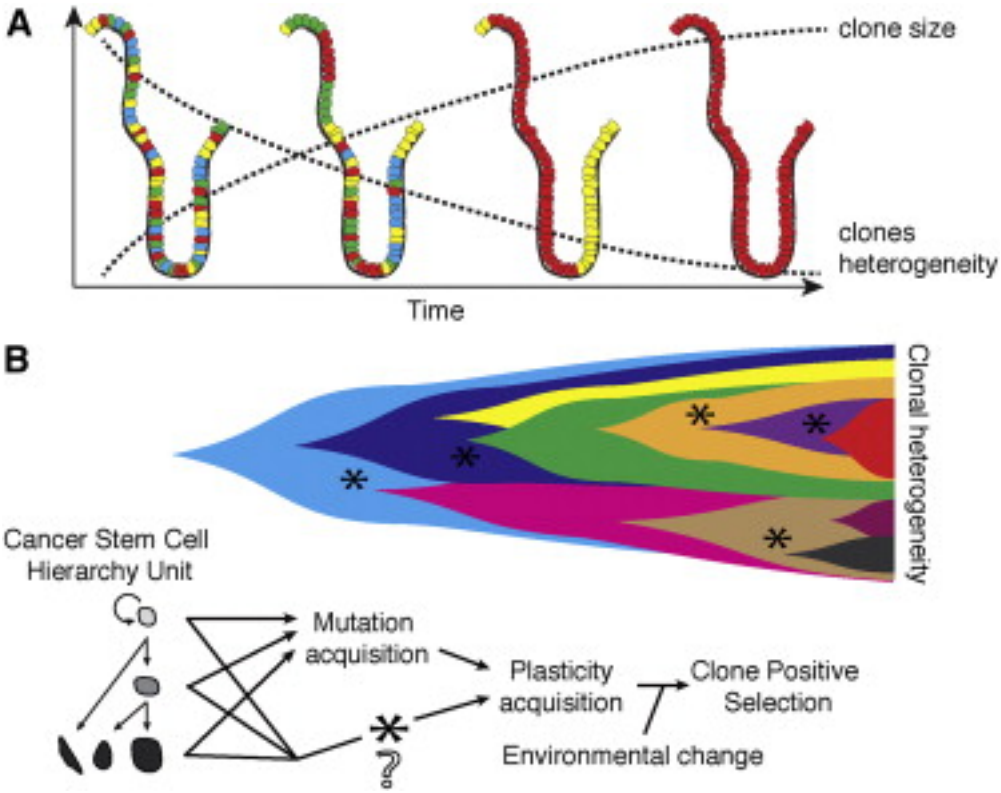

Figure 3. Clonal heterogeneity in homeostasis and cancer. (A) Multicolor genetic labeling of epithelial cells in the intestine shows neutral competition between stem cells. Over time the number of clones decreases while the clone size increases. (B) Concept that the mechanisms of stem cell plasticity during tissue repair underlie the evolutionary dynamics of cancer stem cell clones. Upper panel: clonal evolution resulting in clonal heterogeneity over time. Lower panel: in tumours clones of cancer stem cells with genomic mutations that confer a growth advantage will expand. In addition changes of the niche (question mark)

could promote clonal evolution without genetic mutation by conferring cell plasticity, as occurs during repair of healthy tissue.

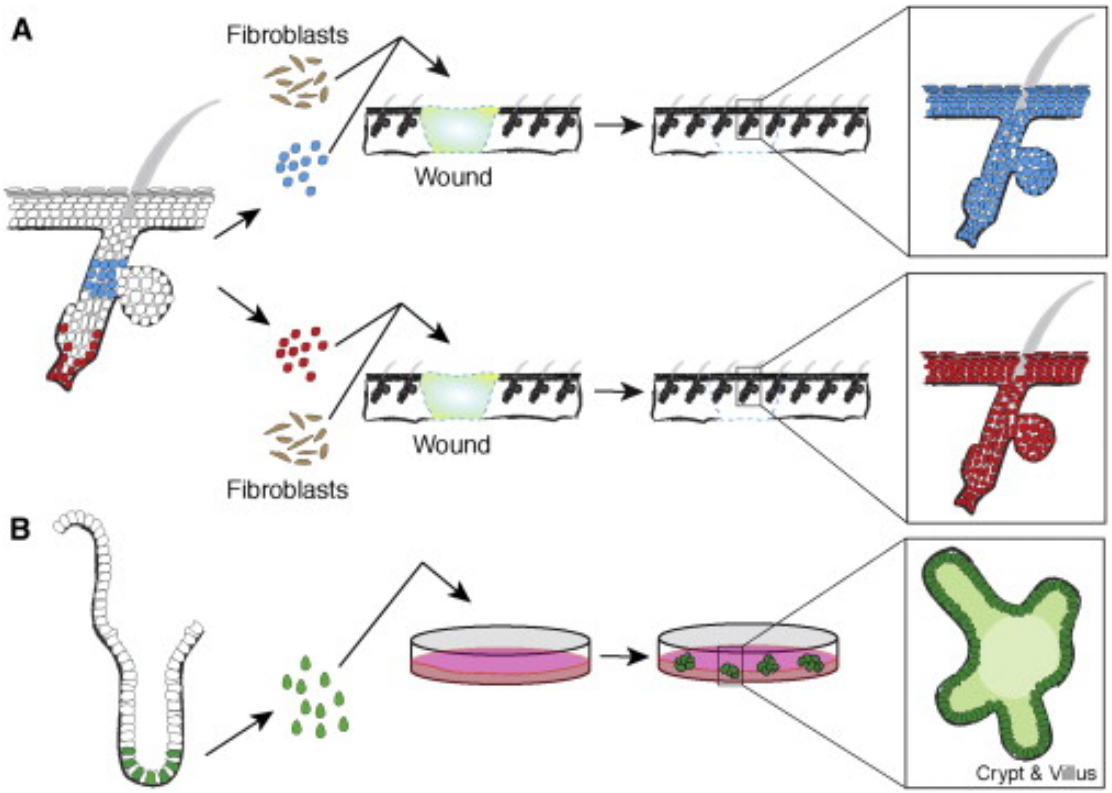

Figure 4. Stem cell plasticity in tissue reconstitution assays. (A) In undamaged epidermis Lgr5 (red) and Lrig1 (blue) stem cells contribute to different regions of the tissue. However, when disaggregated and combined with fibroblasts both stem cell populations can reconstitute all the lineages of the epidermis. (B) Lgr5-positive intestinal stem cells (green) are sufficient to generate crypt-villus units in culture.

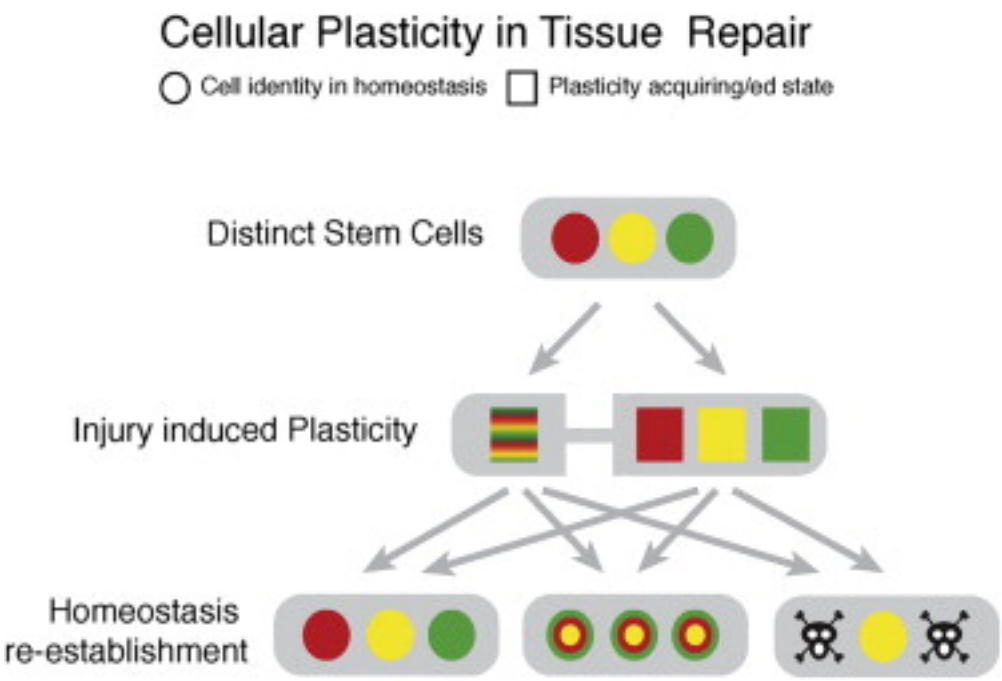

Figure 5. Model of stem cell plasticity during repair of injured tissue. Three different stem cell populations are indicted by red, orange and green. circles The plastic state induced by injury (rectangle) could either be common to all stem cell types (stripes) or different for different stem cell types. Once homeostasis is re-established, stem cells could revert to their original state (red, yellow, green circles), or acquire a new identity (multi-colored circles). Alternatively only a subset of stem cells could contribute to the repaired tissue, the others undergoing cell death.

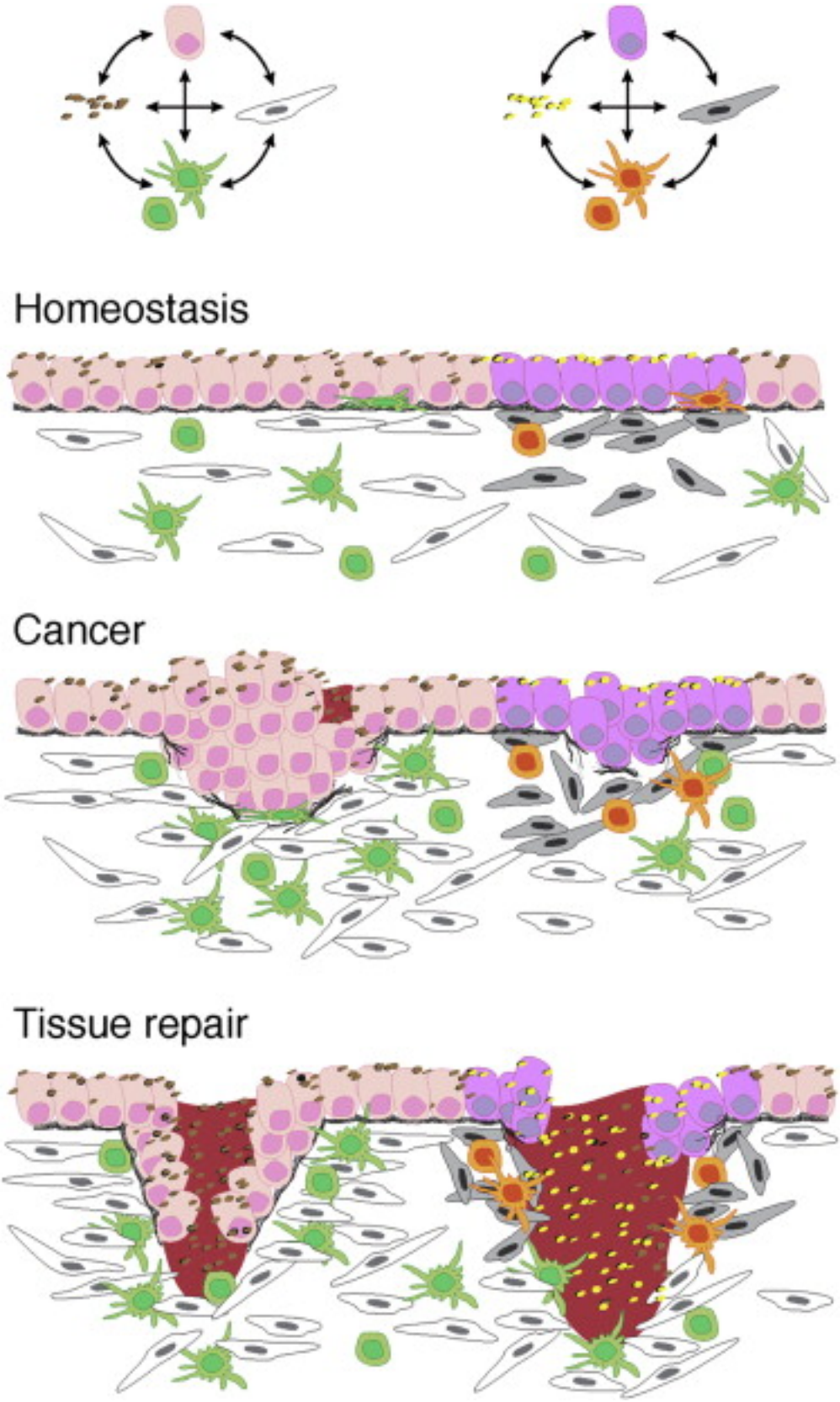

Figure 6. Fibroblasts, immune cells and bacteria as components of the epithelial stem cell niche during homeostasis, tissue repair and cancer. Two different epithelial compartments within one tissue are shown (pale and dark pink). Each

type of stem cell resides in a different niche (bacteria brown or yellow, fibroblasts white or grey, immune cells green or orange). Double headed arrows indicate crosstalk between stem cells and niche cells. In homeostasis the different niche constituents may reinforce different stem cell identities. In tissue repair and cancer the different niches may influence stem cell behavior, such as proliferation and cell migration.

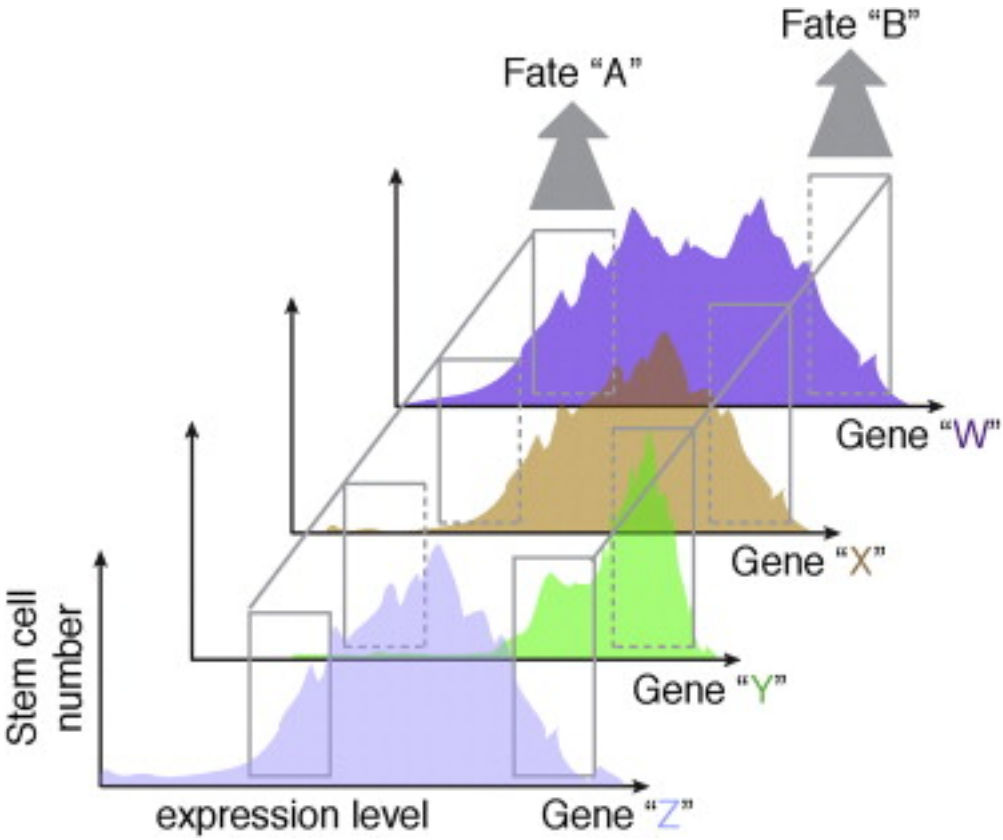

Figure 7. Transcriptional noise as an epithelial stem cell fate regulator. Stochastic variation in the expression levels of a single gene or several co-expressed genes are represented as a mechanism to control cell fate decisions. Variation in the amplitude of transcriptional noise is within a set of genes is represented as an additional mechanism influencing stem cell fate.

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